

We Claim:

1. A method for identifying a compound that inhibits an enzyme having an ATP binding site comprising the steps of:
 - 5 (a) contacting a composition comprising the enzyme having an ATP binding site, an analyte capable of binding to the ATP binding site of said enzyme, and a test compound, and
 - (b) detecting whether said test compound inhibits said analyte from binding said ATP binding site.
- 10 2. The method of claim 1, wherein said enzyme is a kinase.
3. The method of claim 1 wherein said test compound is a competitive inhibitor of said analyte.
- 15 4. The method of claim 1 wherein said analyte is p-fluorosulfonylbenzoyl 5'-adenosine (FSBA).
5. The method of claim 4, wherein the FSBA is biotinylated.
- 20 6. The method of claim 1, wherein the enzyme comprises a conserved lysine in the ATP binding site.
7. The method of claim 6, further comprising binding the analyte to the conserved lysine.
- 25 8. The method of claim 1 wherein said detecting step comprises using mass spectrometry.
9. The method of claim 1, wherein said detecting step comprises using a protease assay.
- 30 10. The method of claim 1, wherein said detecting step comprises using a kinase assay.
11. The method of claim 1, wherein said detecting step comprises using Western blot.
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12. A method for identifying a compound that inhibits a kinase having an ATP binding site comprising the steps of:
- 5 (a) contacting a composition comprising a kinase and an analyte that binds to an ATP binding site of said kinase,
- (b) detecting binding of said analyte to said ATP binding site,
- (c) contacting a composition comprising said kinase, said analyte, and a test compound, and
- 10 (d) detecting whether said test compound inhibits said analyte in step (c) from binding said ATP binding site.
13. The method of claim 12, wherein the kinase comprises a conserved lysine in the ATP binding site.
14. The method of claim 13, further comprising binding the analyte to the conserved lysine.
- 15 15. The method of claim 12, wherein said detecting step comprises using mass spectrometry.
- 20 16. The method of claim 12, wherein said detecting step comprises using a protease assay.
17. The method of claim 12, wherein said detecting step comprises using a kinase assay.
- 25 18. The method of claim 12, wherein said detecting step comprises using Western blot.
19. The method of claim 12, wherein said test compound is a competitive inhibitor of said analyte.
- 30 20. The method of claim 12, wherein said analyte is p-fluorosulfonylbenzoyl 5'-adenosine (FSBA).
21. The method of claim 20 wherein the FSBA is biotinylated.

22. A method for identifying a test compound that inhibits a kinase having an ATP binding site comprising the steps of:
- 5 (a) contacting a composition comprising the kinase and test compound,
(b) contacting a composition comprising said kinase and said test compound with an analyte, and
(c) detecting whether said test compound inhibits said analyte in step (b) from binding said ATP binding site.
- 10 23. The method of claim 22, wherein the kinase comprises a conserved lysine in the ATP binding site.
24. The method of claim 23, further comprising binding the analyte to the conserved lysine.
- 15 25. The method of claim 22, wherein said detecting step comprises using mass spectrometry.
26. The method of claim 22, wherein said detecting step comprises using a protease assay.
- 20 27. The method of claim 22, wherein the said detecting step comprises using a kinase assay.
28. The method of claim 22, wherein said test compound is a competitive inhibitor of said analyte.
- 25 29. The method of claim 22, wherein said analyte is p-fluorosulfonylbenzoyl 5'-adenosine (FSBA).
- 30 30. The method of claim 29, wherein said FSBA is biotinylated.
31. A method for identifying a protein kinase having an ATP binding site comprising the steps of:
- 35 (a) contacting a composition comprising the protein kinase with an analyte capable of binding said kinase, and

(b) detecting whether analyte binds to said kinase.

32. The method of claim 31, wherein said analyte is p-fluorosulfonylbenzoyl 5'-adenosine (FSBA).

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33. The method of claim 32, wherein the FSBA is biotinylated.

34. The method of claim 31, further comprising contacting said protein kinase with a kinase inhibitor.

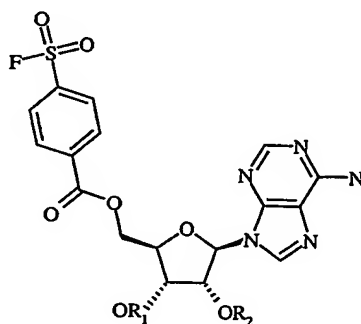
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35. The method of claim 31, wherein said detecting step comprises using Western blot.

36. The method of claim 31, wherein said detecting step comprises using LC/MS.

15 37. A compound of Formula I

(I)

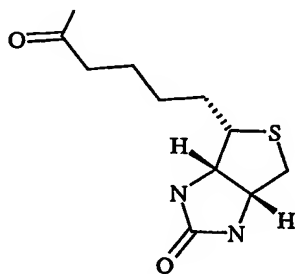


where

R₁ and R₂ are independently H or biotin as represented by Formula II:

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(II)



38. A process for making a compound of Formula I comprising the steps of:
a) dissolving (+)-Biotin, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate in dry N,N-dimethylformamide and heating;
5 b) cooling the solution of step a);
c) adding di-isopropylcarbodiimide to the solution of step b); and
d) adding the solution of step c) to an ice-cold solution of 5'-(4-fluorosulphonylbenzoyl) adenosine 1DMF and diisopropylethylamine; and
e) adding N,N-dimethylaminopyridine in dry DMF to the solution of step d)
10 and warming slowly.
39. A process for making a compound of Formula I comprising the steps of:
a) dissolving (+)-Biotin, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, N-hydroxybenzotriazole·H₂O, N,N-dimethylaminopyridine and 5'-(4-fluorosulphonylbenzoyl) adenosine
15 in dry N,N-dimethylformamide;
b) chilling the solution of step a);
c) adding N,N-diisopropylethylamine to the solution of step b); and
d) warming the solution of step c) to room temperature and stirring;
40. A method for identifying the mode of action of a test compound that inhibits a first enzyme having a first ATP binding site comprising the steps of:
20 (a) contacting a composition comprising the first enzyme having a first ATP binding site, an analyte capable of binding to the first ATP binding site of said enzyme, and a test compound, wherein the ratio of concentration of analyte to test compound is at least 1:1; and
25 (b) detecting whether said test compound inhibits said analyte from binding said first ATP binding site, wherein diminution of analyte binding to the first ATP binding site indicates competitive inhibition by said test compound.
41. The method of claim 40, further comprising:
30 (a) contacting a composition comprising a second enzyme having a second ATP binding site, an analyte capable of binding to the second ATP binding site of said second enzyme, and said test compound;
(b) detecting whether said test compound inhibits said an analyte from binding said second ATP binding site; and
35 (c) determining if said test compound is a selective inhibitor of said enzyme.

42. The method of claim 41, wherein said test compound that diminishes binding of said analyte to said first enzyme but does not diminish binding of said an analyte to said second enzyme is a selective inhibitor of said first enzyme.